Nigerian Journal of Engineering Science Research (NIJESR).
Vol. 6, Issue 1, pp.26-36, March, 2023

ISSN: 2636-7114
Journal Homepage: https://www.iuokada.edu.ng/journals/nijesr/

Evaluating the Effects of Process Conditions on Bioethanol Production from Raffia Palm Fronds

[1*]Yerima Yakubu, [2]Abdurrahman Muhammad Isa, [3]Khamis Alamin Lamido, [4]Sani Gaurav

[1]Department of Chemica/Petroleum Engineering, Igbinedion University, Okada, Edo State, Nigeria
yerima.yakubu@iuokada.edu.ng
[2]Department of Civil Engineering Sharda University, Greater Noida, India
mabdurrahman63@gmail.com
[3]Department of Civil Engineering, Sharda University, India
gauravsaini@gmail.com
[4]Department of Civil & Environmental Engineering, Netaji Institute of Technology, India
alaminkhamis@gmail.com

**Corresponding Author:* yerima.yakubu@iuokada.edu.ng (08055699772)

Manuscript History
Received: 28/11/2022
Revised: 19/01/2023
Accepted: 12/02/2023
Published: 30/03/2023

Abstract: *Biofuels have recently been gaining interest as a potential substitute for fossil fuels due to the inherent environmental and economic challenges associated with the latter. This study evaluated the potential of Raffia palm fronds for bioethanol production using Trichoderma reesei as a source of cellulase. The impacts of temperature, pH, and substrate concentration on ethanol concentration were investigated. A physical and biological pretreatment combination was used for effective hydrolysis and fermentation. The results show that ethanol yield is greatly affected by pH, temperature, substrate concentration, and fermentation time. High productivity is obtainable at a temperature of 40^{0}C at 40 hours of fermentation. This implies that higher sugar conversion to ethanol using Trichoderma reesei is favoured by this temperature. However, at higher temperatures, a decrease in ethanol yield was observed, which may be attributed to the low heat tolerance of the yeast, leading to a decreased activity and subsequent denature. pH alterations exerted a more significant effect on ethanol yield. A pH of 4.5 produced the highest yield at 24 hours. Beyond this period, the concentration sharply decreased due to more ethanol which replaces water, thus inhibiting the enzymes' activity. Substrate concentration also influences productivity, and the highest yield was obtained at a substrate concentration of 100 g/l. A maximum yield of 54 g/l was obtained at a temperature of 40 ^{0}C and a substrate concentration of 100g/l, with the pH set at 4.5.*

Keywords: *Bioethanol, Raffia palm fronds, Trichoderma reesei, Pretreatment, Enzymatic hydrolysis, fermentation*

INTRODUCTION

The continuous growth in the world's population and the advancement in the industrial sector has resulted in a significant increase in energy demand. Fossil fuels cover about 80% of the global energy demand (EESI, 2021), which has led to a considerable decrease in fossil fuel energy reserves and more carbon footprints associated with the increasing rate of fossil fuel-based energy consumption.

These problems, coupled with the instability of oil prices, have catalyzed more interest in renewable, healthier, sustainable, and environmentally friendly alternatives (Antizar-Ladislao & Turrion-Gomez, 2008). Renewable energy sources, such as biofuel, have been gaining more interest during the last few decades. Biofuel based energy can serve as a potential alternative and could be used to address most of the current challenges associated with fossil fuel (Dahnum *et al.*, 2015). With the rapid technological development, several techniques have been developed over the recent years, often in wartime, making the conversion more efficient. Nevertheless, the main objective now is to upgrade the production to be cost-competitive in today's markets (Ogbuagu, 2008). Bioethanol is ethyl alcohol with a molecular formula of C_2H_5OH produced by microbial fermentation (contrary to alcohol produced by the petrochemical process), which can be used as fuel for transportation. It has a density of 0.789 kg/l, a molecular mass of 46.07 g/mol, a boiling temperature of 78.4^0C and an octane number of 99 (Australian Government, 2022). The high octane bioethanol has provided the same anti-knocking effect with aromatics derived from petroleum such as benzene (Walker, 2011). According to Chin and H'ng (2010), bioethanol is a clear, colourless, biodegradable alcohol produced by the fermentation of sugars (e.g. glucose, sucrose, etc.) obtained from plants with the use of microorganisms (yeast or bacteria). It has low toxicity and produces relatively low carbon footprints. Bioethanol feedstock contains either starch (e.g. corn, cassava, etc.), sucrose (e.g. sugar beet, sugarcane, etc.), or lignocellulose (e.g. sugarcane bagasse, palm fronds, wood and straw, etc.) (Chin *et al.*, 2010). However, only sugarcane, cassava, and corn starch have been produced industrial.

More than 75% of the bioethanol generated globally is used for automobiles. It is used as liquid fuel for internal combustion engines, either neat or blended in petrol. The most common gasoline blend is (E10) 10% ethanol and 90% gasoline. However, in 1976, the government of Brazil made it compulsory to blend with 5% ethanol (E5). Since then, it gradually increased to 25% (E25) in 2007. Other bioethanol uses include; additives in paints and cosmetics (Chin & Hng, 2013). Many countries are now shifting towards bioethanol from various plants and plant materials. For example, Brazil uses sugarcane as a primary raw material in bioethanol production. The United States is also utilizing corn for the production of the same. The United States is the largest bioethanol producer, followed by Brazil, producing about 35% of the global production (Chin & Hng, 2013). As the largest global exporter of the same, Brazil exported more than nine hundred million gallons of bioethanol, accounting for about fifty per cent of the total exports in the world (Ibeto *et al.*, 2011). In Nigeria, ethanol production for transportation hasn't yet transited from the level of research and development. Despite a statement by the Nigerian National Petroleum Corporation (NNPC) in 2007, for a planned yearly production of ethanol worth about 150 USD, a commercially large scale production is yet to be recorded (Oloruntoba & Adekanye, 2019). The Production trend of bioethanol can be grouped into three generations. The first-generation biofuels are composed of bioethanol production from agricultural food crops (Carmo *et al.*, 2013). It consists of sugar-containing crops such as sugarcane, whey, sugar beet, molasses, and starchy food crops like maize, wheat, corn, etc. or root crops like cassava. The main setback with this feedstock type is competition with food and land (Dahnum *et al.*, 2015). Despite having high productivity, first-generation bioethanol presents a potential problem of food scarcity, leading to a rise in the cost of living. Second-generation biofuels are produced from non-food agricultural biomass, also known as Lignocellulose biomass, such as stalks of rice, wheat, corn, wood, and other agricultural waste. They are primarily made of Cellulose, Hemicellulose, Lignin, and other minor components. Lignocellulose materials are one of the most abundant biopolymers on earth. Lignocellulose feedstock accounts for about 50% of the world's total biomass, with an estimate of 10 to 50 million tons of annual production (Claassen *et al.*, 1999).

Second-generation biofuels are associated with enormous benefits in the sense that they 1) decrease net carbon emission, 2) reduce energy dependency, 3) increase energy efficiency, and 4) simultaneously overcome the limitation inherent in the first-generation biofuels (Antizar-Ladislao & Turrion-Gomez, 2008). The highest recorded conversion of second-generation bioethanol was with switchgrass accounting for approximately 80% conversion efficiency (201L/t) (Chin & Hng, 2013). It is important to note that second-generation bioethanol is associated with a more complex production process than the first generation due to the additional effort required to remove the lignin content of the biomass (Dahnum *et al.*, 2015). The third-generation biofuels are obtained from algae, associated with a distinctive growth yield on relative terms. They are regarded as a valuable substitute for fossil fuel and can outweigh the performances of first and second-generation biofuels. However, large scale production of this form of biofuel is currently a significant challenge yet to be overwhelmed. This sector's research and development are still evolving without an effective breakthrough (Brennan & Owende, 2010). EXXON. Mobil invested a sum of six million USD into the research and development of algal biofuel in 2008. It concluded that biofuels derived from algae would not be a feasible option for another 25 years. Lignocellulosic biofuels are the most promising feedstock for bioethanol production, considering their high availability and low cost (Dahnum *et al.*, 2015). Raffia palm (lignocellulose biomass) tree is one of the enormous palms in Africa, primarily found in the tropical rain forest. It is regarded as a native of the *Palmae* or *palmacea* family. Close to thirty species of them can be found in the tropical and subtropical parts of the world (Doncel, 2019). Ismail *et al.* (2014) reported that palm frond contains on average 40-50 %, 20-35 %, and 15-35 % of Cellulose, Hemicellulose, and Lignin, respectively. Wine production from Raffia contains 37.6% (Obot, 2000), >40% (Ire *et al.*, 2020) and 30-60% (Ire *et al.*, 2020) alcohol contents. This implies that a reasonable ethanol yield could be achieved upon scaling up. Raphia palm has many uses in various disciplines. Its sap is used for producing traditional wine. The oil extracted from its nut is edible and can be used for cosmetics (Obahiagbon & Obahiagbon, 2009). The lignin component in the plant material creates recalcitrance and hinders cellulose conversion to fermentable sugars. Pretreatment aims to destroy the lignin content in the feedstock, thus exposing the lignocellulose substrate and increasing its surface area and porosity, providing room for effective hydrolysis and fermentation (Dahnum *et al.*, 2015). Pretreatment can be achieved using chemical/physiochemical methods such as explosion techniques (e.g. ammonia fibre explosion, carbon dioxide explosion, etc.), Alkali treatment (e.g. using caustic soda), Acid treatment (e.g. using strong or dilute acid.), Gas treatment (chlorine dioxide, nitrogen dioxide, sulfur dioxide, etc.), Solvent extraction of lignin (e.g. ethanol-water extraction, benzene-water extraction, using swelling agents, etc.) or by adding oxidizing agent (e.g. hydrogen peroxide, ozone, etc.). It can also be achieved via biological/enzymatic processes using fungus or actinomycetes (Salles-Filho *et al.*, 2016). The pretreated samples can be easily subjected to hydrolysis. Hydrolysis converts the polysaccharide sugars in the feedstock to fermentable (monosaccharide) sugars which are then fermented to produce ethanol. Hydrolysis may be chemical (using acid or base or biological using enzymatic reactions (Salles-Filho *et al.*, 2016). One of the concerns on the utilization of ethanol as fuel is the issue of engine design and compatibility. For example, as of 2017, according to IHS. Markit, only about 21 million out of almost 150 million vehicles were Flexible Fuel Vehicles (FFVs) that can operate on an E85 blend in the United States. Furthermore, the shift from fossil fuel to a biofuel-based energy option will require an increase in the cultivation and production of lignocellulose biomass to meet the global demand. Thus, the need for enough land for the cultivation and provision for the cultivation and provision of biorefineries. Kumar *et al.* (2012) argue that for a reasonable decrease in gasoline use as transportation fuel, it is fair to assume that millions of land hectares would be required for mass production.

MATERIALS AND METHODS

All the equipment used were available at the University of Benin Biochemical Laboratory and were approved by the university. They include Miller machine, Erlenmeyer flask (250ml and 1000ml), Cloth sheets (Filter), Sterile disposable Petri dishes, Objectives lenses (X4 and X40 – Olympus and Heto Models), Pipette (25ml), Soil Auger (SEA43/SEA438), Water bath (Dargatz, 6640-12-198-6544), Shake flask (250 ml), Measuring cylinder (250ml), Test tube (50ml), Beakers (200ml), Thermometer (Liquid in glass), Plate spreader, Latex hand gloves, pH meter (Hanna Instrument, China), Gallen Kamp incubator, Centrifuge (Cence L550, China), Specimen Bottle (Amber colour), Coverslip (20mm, 4/5" wide), Whatman Filter Paper, and Weighing balance (Scout Pro – 400g).

The chemicals/reagents used were analytically graded. They include; Ethylene diamine tetraacetate (18.61g), glycol monoethyl ether (10ml), Disodium phosphate anhydrous (4.56g), N-octanol ($C_8H_{18}O$) (few drops), Cetyl trimethyl ammonium bromide (20g), Sulphuric Acid (1M) and Acetone.

2.1 Preparation of Sample

One kilogram (1kg) of Raffia Palm Fronds (RPF) was obtained from a farm in Ovia North East, Okada, Edo State of Nigeria. The RPF (Fig. 1) obtained was sun-dried for three (3) days and then chopped into smaller pieces to enhance the accessible surface area before being taken to the University of Benin Biochemical Laboratory, Edo State, for analysis.

Fig. 1 Sun Drying of Raffia Palm fronds

2.2 Physical and Enzymatic Pretreatment

2.2.1 Milling pretreatment

The sun-dried and crushed RPF sample (Fig. 2) was dried again in a Galen Kamp incubator at 45^0C for five days for the palm branch stock and seven days for the palm leaves. After that, they were separately loaded on the miller machine at 30 revolutions per minute in the presence of porcelain pebbles of various sizes.

Fig. 2 Grinded Sample

2.2.2 Enzyme Pretreatment

500grams of the milled oil palm branch stock and palm leaves materials (differently) were put into a 1000ml Erlenmeyer flask. It was moistened with 800ml of crude cellulase enzyme and then put into the incubator at 50^0C for 5 hours. After mixing the solid material well with distilled water (100ml), it was filtered with cloth sheets to separate the content into liquid and solid parts. The filtered liquid was put in a centrifuge at 10,000 revolutions per minute and then transferred into a 250mls Erlenmeyer flask for fermentation.

2.3 Maintenance of Micro Organism

The organism Trichoderma reseei was obtained and cultured on potato dextrose agar. It was incubated at 25^0C for five days until the organism sporulated. The culture was then kept at 4^0C until when needed.

2.4 Biological Pretreatment Process using Fungal Culture

The culture kept earlier was then re-cultured on potato dextrose agar in Petri-dishes. Distilled water (sterilized) was added to the conical flasks containing the finely grinded sample. The sample was then put in an autoclave at 121^0C for about 15 minutes at 15 psi (Chin & H'ng, 2010), and the pH was adjusted to 5.6 from 4.5, which is the optimum pH for fungal growth. The sample in the conical flask is then inoculated with 12ml of fungal culture, mixed thoroughly, and then incubated at room temperature. The conical flasks were then wrapped with aluminium foil.

2.5 Extraction of Fermentable Sugar

The crude fermentable sugar (Fig. 3) from fermentable materials was extracted by a simple contact method. 40g of pretreated RPF is dissolved in 1 litre of distilled water. The content is appropriately mixed every twenty minutes for two hours at 25^0C. The suspension obtained after mixing was then filtered and centrifuged for twenty minutes at 4,0000 revolutions per minute to obtain a clear crude fermentable sugar. The concentrated fermentable sugar was then used as a fermentation medium source for bioethanol production.

Fig. 3 Sugar samples ready to be fermented

2.6 Fermentation Process

At the beginning of the fermentation process, the untreated samples in the conical flask were set to pH 4.5. Then, 20g of yeast granules were added to the pretreated samples and mixed properly. The conical flask was then entirely covered in the dark between 0-120 hours for fermentation to make the system anaerobic. Samples were collected and tested at intervals of 24 hours, and the fermentation step was carried out at a varying temperature, pH, and substrate concentration.

The temperature was varied between 25^0C and 40^0C, the optimal temperature range for Fermentation (Lomthong *et al.*, 2021). A pH controller that uses sodium hydroxide and sodium chloride was used to vary the pH through 4.0, 4.5, 5.0, 5.5, and 6.0. A standardized pH meter (Hanna Instrument, China) was used to measure the pH values. The results for the optimum pH (4.5) and temperature (40^0C) were used to conduct the fermentation at varying glucose concentrations, from 20g/l to 100g/l. It is important to note that all experiments were conducted in duplicates.

2.7 Distillation Process

The distillation was carried out via the soxhlet apparatus. The evaporated ethanol was condensed into an inner vessel of the Soxhlet apparatus.

2.8 Ethanol Measurement based on Specific Gravity

25 ml of the fermented samples were collected and diluted with another 25ml of water. It was then taken to the Soxhlet apparatus (Fig. 4) for distillation. The diluted sample was distilled, its specific gravity was determined, and the ethanol content was analyzed.

Fig. 4 Soxhlet Apparatus

RESULTS AND DISCUSSION

The potential of RPF for bioethanol production using *Trichoderma reesei* was studied. The impacts of temperature, pH, and substrate concentration on ethanol concentration were analyzed at varying fermentation times. This was to determine the optimum values of the process parameters at which the highest ethanol yield is obtainable. The changes in ethanol concentration were periodically recorded (from 0 to 120 hours). The experiments were conducted in duplicates, and the averages were used in plotting graphs. The experiment was conducted in four different samples, each for temperature and fermentation time and five different samples for pH, accounting for a total of twenty-six (26) samples (with duplicates) to examine the effect of temperature on ethanol concentration for all three parameters. The concentration graphs formed a bell-shaped structure. After some initial increase, the yield declined with time, providing the optimum values at maximum productivity, which may be because the presence of ethanol reduces water availability, leading to the inhibition and subsequent denature of key glycolytic enzymes.

3.1 Effects of Temperature

The temperature was varied from 25^{0}C to 40^{0}C in four samples, and ethanol concentration was recorded at varying times. It was evident from the experimental result that fermentation temperature has a considerable effect on ethanol concentration. Figure 5 depicts that high productivity is obtainable at a temperature of 40^{0}C between 20 and 40 hrs, which implies that this temperature favours a higher conversion of sugar to ethanol using *Trichoderma reesei*. This can be attributed to *Trichoderma reesei* being mesophilic. However, at very high temperatures, the productivity of ethanol decreases due to more significant inhibition. Some yeasts may lack tolerance to high heat leading to a decreased activity and subsequent denature (Dimos *et al.*, 2019).

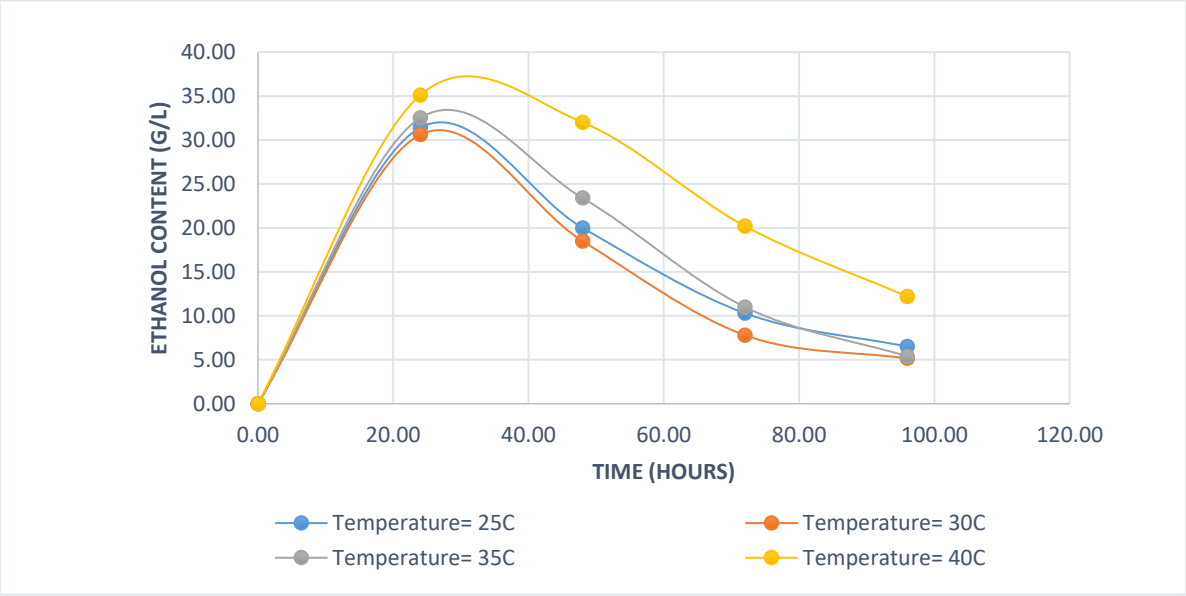

Fig. 5 Temperature effects on ethanol concentration

3.2 Effects of pH

The impact of pH on ethanol production was determined by running the experiment at varying pH values; 4.0, 4.5, 5.0, 5.5, and 6.0, with the temperature set at 35^{0}C. The results show that ethanol production is greatly affected by the fermentation pH. Fig. 6 shows that the highest ethanol concentration can be obtained at an optimum pH value of 4.5.

The impact of pH on ethanol concentration was more significant than the other two variables. The difference between the individual pH curves can be seen in Fig. 6. pH 4.5 produced the highest yield, within a periof of 24 hrs. Beyond this time, the productivity was sharply decreasing due to the availability of more ethanol which replaces water, thus, inhibiting the enzymes' activity and causing their subsequent denature. Ogbonda and Kiin-Kabari (2013) using *Blastomyces* species for cellulase reported higher amounts of ethanol produced at higher acidic (low pH) conditions with highest amount (4.95%) at a pH of 5.0. They also noted that as acidity decreased (higher pH), the amount of ethanol produced decreased with the least (3.03%) at a pH of 7.0. Fig. 6 shows that above a pH of 4.5, the yield decreases sharply to a concentration lower than that obtained at pH 3.5 and 4.0. This implies that the acidic medium favours the high yield production of ethanol. According to (Dimos *et al.*, 2019), more acidic or more basic conditions both inhibit the growth of yeast cellulose. In an acidic medium, the growth of some harmful bacteria are retarded, and that of yeast is favoured. However, yeast produces acid rather than alcohol at a very low pH.

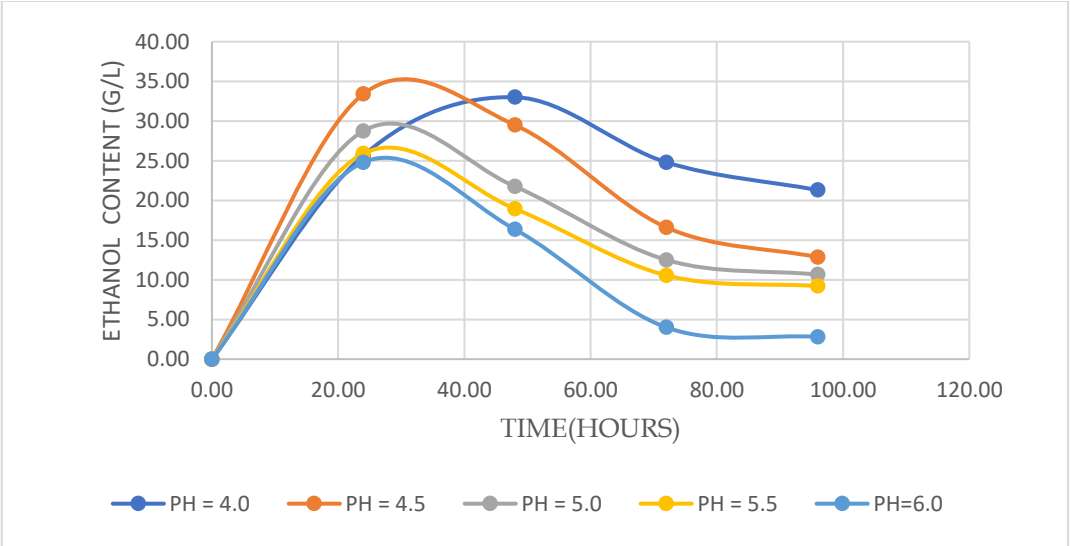

Fig. 6 pH effects on Ethanol concentration with time

3.3 Effects of substrate concentration

The available sugar concentration determines the ethanol production rate (Ohimain, 2012). This may be because the yeast starves at low substrate concentration, resulting in a decline in its productivity. It was observed that higher substrate content leads to higher ethanol concentration due to higher protein utilization by yeast cells. Mardawati *et al.* (2018) reported that the high concentratios of substrate in the medium increase the concentration of the glucose, in which the highest concentration was 3.2g/L. They further noted that the higher the substrate loading resulted in lower hydrolysis yield and hence, ethanol yied. Fig. 7 shows that the highest concentration is obtainable at a substrate concentration of 100 g/l. The graph also shows that, for any concentration, the highest yield can be obtained at about 20 to 40 hours, beyond which the concentration is reduced.

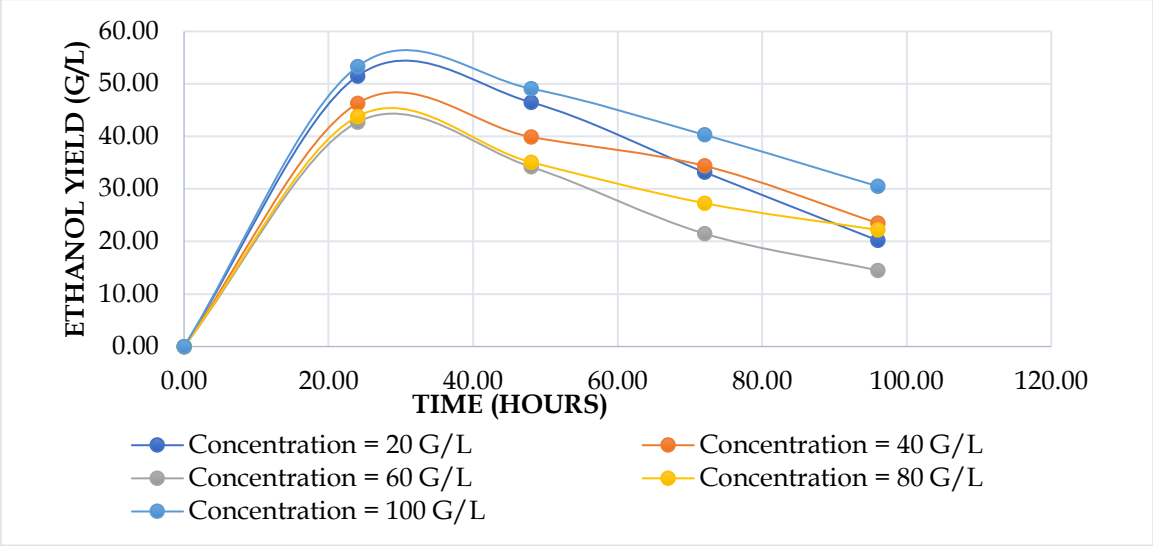

Fig. 7 Substrate concentration effects on ethanol concentration with fermentation time

CONTRIBUTION TO KNOWLEDGE

Bioethanol was successfully produced from Raphia palm fronds using *Trichoderma reesei* as a source of *cellulase.* Raphia palm fronds is a novel substrate in the production of bioethanol with the use of *Trichoderma reesei* as cellulase source.

CONCLUSION

The conversion of RPF to fermentable sugar and its subsequent fermentation to bioethanol was successfully investigated. The results show that RPF can be used as a potential feedstock for bioethanol production because of its high abundance and good conversion efficiency. The effects of temperature, pH, and substrate concentration were also analyzed at varying fermentation times. It was found that these process parameters significantly affect ethanol yield. A temperature of 40^0C was the optimum fermentation temperature at a pH of 4.5 for a fermentation time of 20 to 40 hours. The above results conform closely with the available literature, for which Leghlimi *et al*. (2017) states that a temperature between 35^0C and 40^0C favoured the production of cellulase by the isolated strain, *Trichoderma reesei*. The optimum pH was found to be 4.5 with the temperature been set at 40^0C. A maximum ethanol yield of 54 g/l was observed at a temperature of 40^0C, pH of 4.5, and substrate concentration of 100 g/l. It is recommended that other parameters affecting the fermentation yield be investigated to maximize ethanol yield to the highest possible value. Statistical and artificial intelligence techniques should also be adopted to optimize production. Various organisms and strains should be compared to identify the most effective bioethanol production strain.

CONFLICT OF INTEREST

There is no conflict of interest for this research work.

ACKNOWLEDGEMENT

We sincerely cknowledge the assistance of Prof S. E. Ogbeide for reading through this study and supportive colleagues, we appreciate you.

REFERENCES

Antizar-Ladislao, B., & Turrion-Gomez, J. L. (2008). Second-generation biofuels and local bioenergy systems. In *Biofuels, Bioproducts and Biorefining*. https://doi.org/10.1002/bbb.97

Australian Government. Department of Climate change, Energy, The Environment and water. https://www.dcceew.gov.au/environ. Accessed July, 2022

Brennan, L., & Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. In *Renewable and Sustainable Energy Reviews*. https://doi.org/10.1016/j.rser.2009.10.009

Carmo, J. B. do, Filoso, S., Zotelli, L. C., De Sousa Neto, E. R., Pitombo, L. M., Duarte-Neto, P. J., Vargas, V. P., Andrade, C. A., Gava, G. J. C., Rossetto, R., Cantarella, H., Neto, A. E., & Martinelli, L. A. (2013). Infield greenhouse gas emissions from sugarcane soils in brazil: Effects from synthetic and organic fertilizer application and crop trash accumulation. *GCB Bioenergy*. https://doi.org/10.1111/j.1757-1707.2012.01199.x

Chin, K. L., H'ng, P. S., Wong, L. J., Tey, B. T., & Paridah, M. T. (2010). Optimization study of ethanolic fermentation from oil palm trunk, rubberwood and mixed hardwood hydrolysates using Saccharomyces cerevisiae. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2009.12.036

Chin, K. L., & Hng, P. S. (2013). A Real Story of Bioethanol from Biomass: Malaysia Perspective. In *Biomass Now – Sustainable Growth and Use*. https://doi.org/10.5772/51198

Claassen, P. A. M., Lopez Contreras, A. M., Sijtsma, L., Weusthuis, R. A., Van Lier, J. B., Van Niel, E. W. J., Stams, A. J. M., & De Vries, S. S. (1999). Utilisation of biomass for the supply of energy carriers. In *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s002530051586

Dahnum, D., Tasum, S. O., Triwahyuni, E., Nurdin, M., & Abimanyu, H. (2015). Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. *Energy Procedia*. https://doi.org/10.1016/j.egypro.2015.03.238

Dimos, K., Paschos, T., Louloudi, A., Kalogiannis, K. G., Lappas, A. A., Papayannakos, N., Kekos, D., & Mamma, D. (2019). Effect of various pretreatment methods on bioethanol production from cotton stalks. *Fermentation*. https://doi.org/10.3390/fermentation5010005

Doncel, G. R. (2019). Estado del Arte de la Economía Circular en Colombia. *Journal of Wind Engineering and Industrial Aerodynamics*

Environmental and Energy Study Institute (EESI) Fossil Fuel. https://www.eesi.org/topic/fossil fuel. Acessed July, 2021

Ibeto, C. N., Ofoefule, A. U., & Agbo, K. E. (2011). A Global Overview of Biomass Potentials for Bioethanol Production: A Renewable Alternative Fuel. *Trends in Applied Sciences Research*. https://doi.org/10.3923/tasr.2011.410.425

Ire, F. S., Edio, P. A., & Maduka, N. (2020). Comparative Assessment of the Microbiological and Physicochemical Quality of a Laboratory Brewed' Burukutu' and Commercialized Products Sold in Some Markets in Port Harcourt Nigeria. *European Journal of Biology and Biotechnology*. https://doi.org/10.24018/ejbio.2020.1.5.85

Lomthong, T., Netprasom, P., Kancharu, N., Jitmala, K., Areesirisuk, A., Trakarnpaiboon, S., & Kitpreechavanich, V. (2021). Very high Gravity (VHG) Bioethanol Production Using Modified Simultaneous Saccharification and Fermentation of Raw Cassava Chips with Molasses by Kluyveromyces marxianus DMKU-KS07. *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-020-01257-1

Kumar, M., Goyal, Y., Sarkar, A., & Gayen, K. (2012). Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks. Applied Energy, 93, 193–204

Mardawati, E; Putri, A. V; Yuliana, T; Rahimah, S.; Nurijanah, S; and Hanidah, I, (2018). IOP Conf, Serv,. Earth Environ, Sc. 230 012079. DO1 10.1088/1755-1315/230/1/012079

Obahiagbon, F. I., & Obahiagbon, F. I. (2009). A review of the origin, morphology, cultivation, economic products, health and physiological implications of Raphia palm. *African Journal of Food Science*.

Obot, I. S. (2000). The measurement of drinking patterns and alcohol problems in Nigeria. *Journal of Substance Abuse*. https://doi.org/10.1016/S0899-3289(00)00047-X

Ogbuagu, M. N. (2008). Vitamins, phytochemicals and toxic elements in the pulp and seed of raphia palm fruit (Raphia hookeri). *Fruits*. https://doi.org/10.1051/fruits:2008025

Ohimain. (2012). Traditional Fermentation and Distillation of Raffia Palm Sap for the Production of Bioethanol in Bayelsa State, Nigeria. *Journal of Technology Innovations in Renewable Energy*. https://doi.org/10.6000/1929-6002.2012.01.02.8

Oloruntoba, A., & Adekanye, P. K. (2019). Biofuel Development in Nigeria Production and Policy Challenges. *Journal of Energy Research and Reviews*. https://doi.org/10.9734/jenrr/2019/v3i330100

Salles-Filho, S. L. M., Cortez, L. A. B., da Silveira, J. M. F. J., & Trindade, S. C. (2016). Global Bioethanol: Evolution, Risks, and Uncertainties. In *Global Bioethanol: Evolution, Risks, and Uncertainties*. https://doi.org/10.1016/C2014-0-03857-9

Ogbonda, K.H and Kiin - Kabari (2013). Effect of temperature and pH on ethanol production by Blastomyces species isolated from the intestine of oil palm weevil (Rhynchophorus palmmarun, Coleoptera). African Journalof Biotechnology. 12 (6) 588 - 591. DOI:10.5897/AJB 10.1477

Walker, G. M. (2011). 125th anniversary review: Fuel alcohol: Current production and future challenges. *Journal of the Institute of Brewing*. https://doi.org/10.1002/j.2050-0416.2011.tb00438.x